Molecular neuro-oncology in clinical practice: a new horizon

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Molecular neuro-oncology entering clinical practice: a new horizon

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[1]Abbreviations: ATRX, α-thalassemia/mental-retardation-syndrome-X-linked; CDKN, cyclin-dependent kinase; CIC, *Drosophila* homolog of capicua; EORTC, European Organisation for Research and Treatment of Cancer; FISH, fluorescence in situ hybridization; FUBP1, far upstream element (FUSE) binding protein; G-CIMP, Glioma CpG island methylator phenotype; H3F3A, H3 histone, family 3A; IDH, isocitrate dehydrogenase; MGMT, O^6-methyl-guanine-DNA

methyltransferase; MAPK, mitogen-activated protein kinase; NCIC, National Cancer Institute of Canada; NOA, Neurooncology Working Group of the German Cancer Society; PCR, polymerase chain reaction; PCV, procarbacine, CCNU, vincristine; PET, positron emission tomography; PFS, progression-free survival; PTEN, phosphatase homolog on chromosome ten; RT, radiotherapy; RTK, receptor tyrosine kinase; RTOG, radiation therapy oncology group; TMZ, temozolomide; TRAF, tumour necrosis factor (TNF) receptor-associated factor; WHO, World Health Organization.

Abstract

Primary brain tumours are heterogeneous regarding histology, genetics, and outcome. Although the World Health Organization (WHO) Classification of Tumors of the Central Nervous System has greatly aided in standardizing diagnostic criteria throughout the world, it does not yet consider the tremendous progress made recently in the molecular classification of many brain tumours. Recent practice-changing academic clinical trials have defined a role for routine assessment of *MGMT* promoter methylation in glioblastoma of the elderly and 1p/19q co-deletions in anaplastic oligodendroglial tumours. Moreover, large scale molecular profiling approaches have identified new mutations in gliomas, affecting isocitrate dehydrogenases (*IDH*) 1 and 2, *H3F3*, *ATRX* and *CIC*, and allowed to subclassify gliomas into distinct molecular subgroups with characteristic features of age, localization, and outcome, although they do not yet predict benefit from therapeutic interventions. Similarly, transcriptome-based classification of medulloblastoma has delineated four variants that may now be candidate diseases to explore novel targeted agents.

Search Strategy and Selection Criteria section

References for this review were identified through searches of PubMed with the search terms „brain tumo(u)r", "glioma", "medulloblastoma", "meningioma", "ependymoma", "molecular", "predictive", and "prognostic" in various combinations, from 2000 to January 2013. Articles were also identified through searches of the authors` own files. Only papers in English were reviewed. Data available only

in Abstract form were not included. The final reference list was generated on the basis of originality and relevance to the broad scope of this review.

Introduction

The World Health Organization (WHO) Classification of Tumours of the Central Nervous System distinguishes tumours by histological criteria and, based on morphological features of anaplasia, additionally allocates a malignancy grade ranging from WHO grade I to IV to each tumour, if applicable. Traditionally the nomenclature of brain tumours is often assigned based on a presumed cell of origin which is mainly deduced from cytological similarities of the tumour cells with the various normal cell types occuring in the central nervous system and its coverings (Webappendix).[1]
From a historical perspective, histopathology thus was the first tool to distinguish brain tumors of different grades of malignancy and (presumed) different histogenetic origin, with the overall goal to provide clinicians with prognostic information. Histopathological classification alone has its limitations, but is greatly aided by immunohistochemical markers that help to discriminate different tumour entities with higher certainty, thereby reducing interobserver variability, and allow for a better characterization of novel tumour entities and variants. A next level of complexity is added by including molecular markers that carry both diagnostic and prognostic information in tumours with histologically similar appearance. Nevertheless, molecular markers have become an integral part of tumour grading and anatomo-pathological assessment in modern neuro-oncology practice because they provide useful information beyond the WHO classification, and molecular marker status now guides clinical decision making at least in subtypes of gliomas.[2] In parallel, several genome- or transcriptome-wide molecular approaches of brain tumour classification indicate that single marker profiling may only be a transient diagnostic standard which may soon be replaced at reasonable cost by tumour genome-wide molecular profiling techniques, including array-based methods as well as diagnostic next generation sequencing. The purpose of this review is to highlight recent advances in the molecular diagnosis and classification of primary brain tumours and to discuss how these advances inform therapeutic decisions.

Gliomas: single marker approaches

IDH mutation

Isocitrate dehydrogenase *(IDH)* mutations, 1p/19q co-deletions, and O^6-methylguanine DNA methyltransferase (*MGMT*) promoter methylation are the three molecular markers that are currently assessed routinely in many brain tumour centres because of their diagnostic, prognostic, or predictive value (Table). *IDH* mutations are early lesions in the development of gliomas and cluster in the active site of theses enzymes at codons 132 of the *IDH1* respectively 172 of the *IDH2* gene. The selective, heterozygous mutational targeting of specific sites of either gene seems necessary and sufficient for neoplastic transformation, suggesting that these mutations confer a gain of function and do not simply affect wildtype IDH function. They favour a neomorphic reaction catalysing the conversion of α-ketoglutarate into D-2-hydroxyglutarate, a candidate oncometabolite accumulating to high concentrations possibly measurable by MR spectroscopy *in situ*[3] and mediating the oncogenic activity of *IDH* mutations.[4] Most interestingly, *IDH* mutations have been reported to be causally linked to profound epigenetic changes, mediated by high concentrations of 2-hydroxyglutarate that inhibit α−ketoglutarate-dependent epigenetic modifiers such as tet methylcytosine dioxygenase (TET) 2, resulting in a glioma CpG island methylator phenotype (G-CIMP).[5] In addition, 2-hydroxyglutarate stimulates hypoxia-inducible factor (HIF) prolyl 4-hydroxylases (EGLN1. 2 and 3) which in turn leads to diminished HIF levels and enhances proliferation as well as soft agar growth of human astrocytes.[6] These insights provided evidence that gliomas with *IDH* mutations have a distinct pathogenetic origin. Hence, the primary molecular approach to classify gliomas of adulthood is to separate gliomas into *IDH*-wildtype *versus IDH*-mutant gliomas. Among the *IDH*-wildtype gliomas, there are distinct entities such as the grade I pilocytic astrocytomas and primary glioblastomas which originate via pathways of tumourigenesis that are independent of the *IDH* pathway and presumably G-CIMP. Conversely, most grade II, grade III and few grade IV gliomas (=secondary glioblastomas) share *IDH* mutations and carry a better prognosis compared to *IDH*-wildtype gliomas of the same histological

grade. In fact, the *IDH* status was a better discriminator of outcome than histological grade in a pooled analysis of 382 WHO grade III and IV-gliomas, excluding oligodendroglial tumours.[7] The prognostic effect of the *IDH* mutation in patients with WHO grade II gliomas appears to be less strong when these patients are not treated with RT or chemotherapy.[8] In fact, *IDH*-wildtype grade II and III gliomas remain poorly characterized groups of tumours that seem to have a less favourable prognosis. Accordingly, the *IDH* status should be incorporated into future brain tumour classifications, especially since the *IDH*-mutant tumours are driven by specific epigenetic alterations, phenotypically characterized as G-CIMP-positive, a status that may be suitable for specific therapeutic interventions that likely will not be successful on an *IDH*-wildtype (G-CIMP-negative) background. For the future development of clinical trials, stratification and separate treatment strategies need to be defined for these distinct subgroups. Pooling *IDH*-mutant and *IDH*-wildtype tumours in the same clinical trials simply because the tumours look alike and are assigned the same histological grade of malignancy is not appropriate anymore.

The *IDH* status is of undisputed diagnostic value, in particular in positively identifying diffuse gliomas and distinguishing them from reactive gliosis as well as various other tumour entities that constitute important histological differential diagnoses but are *IDH*-wildtype lesions. However, the *IDH* status has no defined role in clinical decision making yet within a given tumour entity.

1p/19q co-deletion

Combined losses of chromosomal arms 1p and 19q resulting from an unbalanced t(1;19)(q10;p10) translocation lead to the loss of one hybrid chromosome and thus loss of heterozygosity.[9] This cytogenetic aberration is strongly associated with oligodendroglial histology and rarely found in other tumours. The molecular pathway of oncogenesis associated with this lesion is currently being elucidated: most 1p/19q-co-deleted oligodendrogliomas carry mutations in the *CIC* gene, a homolog of the *Drosophila* gene *capicua*, on chromosomal band 19q13.2[10,11] while *CIC* mutations appear to be less common in 1p/19q-co-deleted oligoastrocytomas.[12] Less frequently there are mutations in the *FUBP1* gene, which encodes the "far upstream element-binding protein", on chromosomal arm 1p.[10,11] 1p/19q-co-deleted tumours have long been known to carry a better prognosis than histologically

indistinguishable tumours of the same grade of malignancy. While it remains controversial whether 1p/19q-co-deleted tumours have a less aggressive natural course, it is well established that they are more sensitive to radiotherapy (RT) or alkylating agent chemotherapy. Long-term results of two large randomized clinical trials – European Organisation for Research and Treatment of Cancer (EORTC) 26951 and Radiation Therapy Oncology Group (RTOG) 9402 – that explored the value of polychemotherapy using procarbacine, lomustin (CCNU) and vincristine (PCV) either prior to or immediately after RT indicate that the inclusion of chemotherapy in the first-line treatment confers a survival advantage which becomes evident after follow-up of more than six years rather specifically in the subgroup of patients with 1p/19q-co-deleted tumours (Table 2). Thus, 1p/19q co-deletions have also predictive value for benefit from chemotherapy, in addition to the characterization of a prognostically more favourable subgroup.[13,14]

The results from these studies led to the suspension of enrolment in the 3-arm CODEL trial which aimed at comparing RT plus temozolomide (TMZ) followed by TMZ (RT/TMZ →TMZ) with RT alone and TMZ alone. This is because RT alone was no longer considered an appropriate treatment for these patients. It has, however, to be noted that these results stem from retrospective analyses and are thus explorative, moreover, it remains unclear how many of the long-term survivors treated with RT plus PCV experience preserved cognitive function and quality of life. Finally, there is controversy whether the same improvement in overall survival could have been achieved with the combination of RT and TMZ or even with alkylating agent chemotherapy alone. The German NOA-04 trial which compared RT and TMZ or PCV alone[15] does not yet provide a conclusive answer regarding differences in long-term disease control with PCV versus TMZ since follow-up was too short at the time of initial publication. Yet, future clinical trials should probably include RT plus PCV polychemotherapy as a control arm.

MGMT promoter methylation

The DNA repair protein MGMT repairs the chemotherapy-induced alkylation at the O^6-position of guanine, the critical mediator of alkylating agent cytotoxicity, and thus counteracts the effects of alkylating chemotherapeutic agents such as nitrosoureas or TMZ. Decreased MGMT protein levels are predicted to result in decreased ability of repair and therefore should be associated with improved

outcome. Hypermethylation of the *MGMT* gene promoter may lead to silencing of the gene and thus decreased protein levels. Numerous clinical trials and cohort studies have shown that *MGMT* promoter methylation is associated with prolonged progression-free and overall survival in glioblastoma patients treated with alkylating agent chemotherapy.[16-22] In the pivotal trial establishing TMZ chemotherapy during and after radiotherapy in newly diagnosed glioblastoma,[23] the benefit from chemotherapy was almost exclusively attributable to patients with a methylated *MGMT* gene promoter.[18,21] In 2012, two independent randomized trials conducted in elderly patients with anaplastic astrocytoma[24] or glioblastoma[24,25] reported a comparison of RT alone versus TMZ chemotherapy alone as initial treatment. Subgroup analyses of both trials demonstrated a superior outcome for chemotherapy in patients with *MGMT* promoter-methylated tumours, but an inferior survival in patients with unmethylated tumours. These results strongly suggest that treatment strategy should be individualised depending on the *MGMT* status when selecting the appropriate treatment for elderly glioblastoma patients who are not commonly treated with combined modality treatment (RT/TMZ→TMZ).

While MGMT determination by immunohistochemistry shows a marked interobserver heterogeneity and does not reliably correlate with promoter methylation or outcome, molecular determination of epigenetic activation status most commonly performed by methylation-specific PCR – or pyrosequencing of bisulfite-modified DNA - has been established as a reliable method. A thorough discussion of the challenges, pitfalls and limitations of *MGMT* promoter methylation analyses has been provided elsewhere.[22] Regarding future developments, it is tempting to speculate that the National Cancer Institute of Canada (NCIC)/EORTC Intergroup trial exploring hypofractionated RT versus hypofractionated RT/TMZ→TMZ may show a survival signal only in patients with *MGMT* promoter-methylated tumours. None of the trials will answer the question whether patients with *MGMT* promoter-methylated tumours may be managed with TMZ alone or might still fare better with RT/TMZ→TMZ.

Anaplastic gliomas, as opposed to the vast majority of primary glioblastomas, show distinct genetic and epigenetic aberration profiles implicating different pathomechanisms of tumourigenesis and progression. Somewhat unexpectedly, but at second thought not surprisingly, a specific predictive value of *MGMT* promoter methylation was not observed in two anaplastic glioma trials where patients

were treated with RT versus alkylating chemotherapy alone[15] or with RT versus RT plus alkylating chemotherapy.[26] Nevertheless, a strong prognostic value of *MGMT* promoter methylation was demonstrated independent of the choice of initial therapy. While it was interesting to observe such a striking difference between anaplastic glioma and glioblastoma regarding the predictive role of *MGMT* promoter methylation, the biological basis of this phenomenon remains to be elucidated.

Interaction of various molecular markers

The three molecular markers described above are not entirely independent. For instance, *IDH*-mutant tumours commonly show *MGMT* promoter methylation, and 1p/19q-co-deleted tumours typically harbour *IDH* mutations.[15,27] *IDH*-mutant/CIMP-positive anaplastic gliomas almost always have the *MGMT* promoter methylated, while the rate of *MGMT* promoter methylation in G-CIMP-negative tumours was 40-50%, similar to primary glioblastoma. Hence, in most anaplastic gliomas, *MGMT* promoter methylation is part of the G-CIMP phenotype while G-CIMP is rare in primary glioblastoma.[28] An exploratory analysis of the NOA-04 trial and validation cohorts from NOA-08 and the German Glioma Network indicated that a methylated *MGMT* promoter status is associated with superior outcome with chemotherapy with or without RT in the absence, but not in the presence, of *IDH* mutations.[29] Thus, *MGMT* promoter methylation may reflect the G-CIMP phenotype of *IDH*-mutant tumours, but may have a different, not yet understood genesis and role in *IDH*-wildtype tumours. Conversely, in a large group of anaplastic glioma patients the epigenetic inactivation of some CIMP-associated genes may sensitize the tumours to RT, and potentially chemotherapy, too, confounding the *MGMT*-related effect. It will be of utmost importance to uncover the identity of such genes and elucidate their biological implications for this phenomenon since they may facilitate the design of new treatment strategies.

Changing treatment paradigms based on biomarker assessment

Figures 1 and 2 summarize how the assessment of *IDH*, 1p/19q and *MGMT* status may be built into a management algorithm for patients with anaplastic gliomas and glioblastoma. Such algorithms are subject to change as new data and concepts emerge and may need to be adapted to institutional

preferences. Importantly, the decision for specific treatments must take into account several issues such as patient preference, tumour location, volume of radiotherapy and potential comorbidities that might increase the risk of toxicity from chemotherapy. Figure 2 does not address the possible role of bevacizumab or other experimental treatments currently explored in the treatment of newly diagnosed glioblastoma.

ATRX mutations

The first evidence for a role of *α-thalassemia/mental-retardation-syndrome-X-linked* (*ATRX)* mutations in gliomas of various grades of malignancy was their association with alternative lengthening of telomeres.[30] It was then shown that *ATRX* mutations are associated with mutations of the *TP53* and *IDH1* genes across glioma entities.[11,31] Most importantly, however, these same studies established *ATRX* mutations to be a very specific marker for astrocytic lineage tumours, including diffuse and anaplastic astrocytomas as well as a subset of oligoastrocytomas, positioning them as an attractive counterpart for 1p/19q co-deletions which appear to be mutually exclusive with *ATRX* mutations. Since the vast majority of mutations detected to date are truncating and thus lead to a reduction of protein levels, immunohistochemical demonstration of loss of ATRX may be a reasonable surrogate marker of *ATRX* mutations. Combining 1p/19q and *ATRX* assessments in a clinical setting may thus help in the future to guide the diagnosis within the spectrum of *IDH*-mutant gliomas and eventually to stratify patients for specific treatments.

H3F3A mutation

Employing exome-wide sequencing of pediatric glioblastomas and pontine gliomas, two recent studies identified frequent mutations in the histone H.3.3 gene (*H3F3A*).[32,33] These mutations cluster at two critical amino acid residues, namely K27 and G34. Interestingly, the two *H3F3A* mutations appear to define distinct epigenetic subgroups of glioblastoma, with *H3F3A* (G34) mutant tumours showing global DNA hypomethylation.[34] Moreover, *H3F3A* mutations are mutually exclusive with *IDH1* mutations, with each *H3F3A* mutation type giving rise to tumours located in separate anatomic compartments and showing differential expression of the transcription factors OLIG1, OLIG2, and

FOXG1.[34] This suggests that pediatric glioblastomas with *H3F3A* K27 or G34 mutations likely arise from distinct cellular origins. Moreover, preliminary clinical correlations suggest that these mutations are associated with distinct clinical outcome, i.e. patients with *H3F3A* K27-mutant tumours appear to show a particularly poor outcome. From a molecular diagnostic point of view, the demonstration of *H3F3A* mutation, e.g., by DNA pyrosequencing, may help to identify different types of pediatric glioblastoma and distinguish these from other gliomas, including pilocytic astrocytoma. More recent data indicate mutations in another gene involved in the regulation of histone methylation in approximately 15% of pediatric and 8% of adult high-grade gliomas, mostly glioblastomas, namely *SETD2*. These mutations were mutually exclusive with *H3F3A* mutations but overlapped in part with *IDH1* mutations in glioblastomas.[35] Collectively, all of these mutations (H3.3G34R/V and SETD2, and H3.3K27M) are believed to directly alter centrally important histone marks such as H3K36 trimethylation and H3.3K27 trimethylation, respectively.

EGFRvIII rearrangement

Approximately 25-30% of primary glioblastomas harbour a characteristic deletion mutant of the *epidermal growth factor receptor* (*EGFR*) gene referred to as *EGFRvIII* which results in constitutive and ligand-independent receptor activity and is considered an important oncogenic mutation. Its prognostic relevance remains controversial, but long-term survival may be inferior in patients whose tumours carry this mutation. *EGFR*-targeted approaches have not been effective in glioblastoma.[36] However, the *EGFRvIII* mutation also creates a new epitope which is immunogenic and thus a candidate tumour antigen in EGFRvIII-positive glioblastoma. Accordingly, vaccination strategies based on this unique peptide sequence have been developed and yielded promising overall survival results in various phase II trials which also provided preliminary evidence for target antigen elimination in recurrent tumours and a link between immune response to the vaccine and outcome.[37,38] A placebo-controlled phase III trial, ACT IV, exploring the efficacy of the EGFRvIII-directed vaccine is currently enrolling patients. Finally, *EGFRvIII* mRNA has also been detected in microvesicles in the serum of patients with *EGFRvIII*-positive glioblastoma,[39,40] indicating that it may serve as a biomarker to monitor response to therapy and detect relapse.

BRAF fusion or point mutation

Tandem duplications of BRAF at 7q34 resulting in KIAA1549:BRAF gene fusions, or sometimes alternative fusion partners, have been recognized as hallmark genetic lesions in pilocytic astrocytoma, with a particularly high incidence in cerebellar pilocytic astrocytomas.[41,42] These fusions are only very rarely found in other tumours. KIAA1549:BRAF gene fusions are therefore considered a very important diagnostic aid to distinguish pilocytic astrocytoma from higher grade astrocytic tumours, a distinction that can be both challenging and therapeutically highly relevant given the fact that pilocytic astrocytomas and glioblastomas share the morphological feature of microvascular proliferation. Other genetic alterations of BRAF including point mutations, in particular the activating $BRAF^{V600E}$ missense mutation, have also been observed in low-grade gliomas as well as grade III/IV gliomas.[43,44] $BRAF^{V600E}$ mutations are particularly common in pleomorphic xanthoastrocytomas, with two thirds of these tumours showing this aberration, which is nowadays easily demonstrated by immunohistochemistry using a mutation-specific antibody.[45] The glioma-associated *BRAF* alterations all exert their oncogenic activity by activating the mitogen-activated protein kinase (MAPK) pathway.[42] More recent studies employing large scale sequencing identified an oncogenic hit in the MAPK pathway in (almost) all pilocytic astrocytomas (Pfister, unpublished) while they did not reveal any significantly mutated gene outside of this pathway, indicating that this tumour may indeed be a single-pathway disease. The availability of small-molecule BRAF kinase inhibitors such as vemurafenib (PLX4032), which specifically targets $BRAF^{V600E}$-mutant tumours, provides a new therapeutic approach to these subgroups of gliomas and preliminary clinical evidence (Pfister, unpublished) suggests that the presence of a $BRAF^{V600E}$ mutation may indeed serve as a potent predictive marker for this subset of patients across gliomas of various grades.

Gliomas: unbiased (high throughput) molecular diagnostic approaches

The notion that high throughput approaches of classifying brain tumours including gliomas are at least a valuable addition, if not superior to histopathological grading has been repeatedly supported in large datasets. However, due to the complexity in data analysis and interpretation, such techniques have not

been introduced into clinical practice (yet). One of the first approaches was to define gene expression signatures derived from classical tumour samples and to use these as an aid to diagnose tumour samples that were less easily assigned to a specific diagnostic entity by histology alone.[46] Gene expression profiling of 276 gliomas resulted in the definition of 7 subgroups that did not simply reflect the histological diagnoses, but were prognostic, and correlated better with survival than histology. In fact, unsupervised bioinformatic clustering added to the prognostic information provided by histology whereas histology did not add to the information obtained by gene expression profiling.[47] The same approach was also applied to patients enrolled in EORTC 26951[13] and confirmed the prognostic value, moreover, it also allowed to identify a subgroup of patients who specifically benefitted from PCV chemotherapy. Superiority of gene expression profiling over 1p/19q testing, however, in predicting outcome was not demonstrated.[48] Gene expression profiling was also used to identify genes associated with outcome and let to the identification of candidate genes such as osteonectin, doublecortin, semaphorin 3B[49] or FABP7.[50] In a subpopulation of 80 glioblastomas from the EORTC/NCIC trial,[23] an expression signature dominated by *HOX* genes was associated with poor survival in patients treated with concomitant chemoradiotherapy, and both the *HOX* signature and *EGFR* expression were independent negative prognostic factors on multivariate analysis.[51] The poor prognostic value of the *HOX* gene-dominated stem cell related self-renewal signature was validated in an independent dataset of the same study. The functional association of the *HOX* gene signature with glioblastoma stem cells has been further substantiated and the negative prognostic effect was confirmed.[52]

In 2006, Phillips and colleagues proposed a new classification of glioblastomas based on supervised gene expression profiling guided by patient outcome and coined the terms of proneural, proliferative and mesenchymal glioblastoma subtypes.[53] Proneural tumours were often anaplastic gliomas, lacked *phosphatase and tensin homolog on chromosome ten* (*PTEN*) or *EGFR* alterations, but exhibited Notch pathway activation, and had a better outcome. The distinction of proliferative versus mesenchymal was less clear, but made on the basis of expression profiles favouring either proliferation or angiogenesis. Extending such analyses, a nine gene set was derived from 4 different data sets which provided independent prognostic information after adjusting for clinical factors and *MGMT* promoter methylation.[54]

Two very influential high throughput studies at the genomic level were published in 2008: first, The Cancer Genome Atlas (TCGA) project demonstrated genetic alterations in three major signalling pathways in the majority of glioblastomas: receptor tyrosine kinase (RTK) / RAS / phosphoinositide-3 kinase (88%), p53 (87%) and retinoblastoma protein (78%),[55] then, *IDH* mutations were discovered in a minority of glioblastoma patients which were young and had a good outcome, consistent with a secondary glioblastoma phenotype.[56] In 2010, a refined expression-based classification suggested the existence of four glioma subtypes: proneural, neural, classical and mesenchymal.[57] Gene expression patterns in these subgroups showed distinct correlations with those of oligodendrocytes, astrocytes, and neurons, providing possible clues to putative lineages of tumour origin. The authors also proposed differential benefit from therapy by subgroup, but these data need to be interpreted with caution, given the retrospective nature of this analysis and the heterogeneous treatments. Interestingly, annotation of the data set with the *MGMT* promoter methylation status revealed that none of the four subgroups displayed an association with the *MGMT* status.[28] Genome-wide DNA methylation profiling provided complementary information and most importantly uncovered the G-CIMP phenotype associated with *IDH1* mutations. Interestingly, *IDH*-mutant and G-CIMP-positive tumours turned out to be a subgroup of the proneural subtype.[58] This discovery was instrumental for uncovering the mechanistic link between *IDH* mutations and genome-wide aberration of DNA methylation.[5] As detailed above, mutations in the histone H3 gene (*H3F3A*) were detected in more than a third of pediatric glioblastomas and more than two thirds of diffuse intrinsic pontine gliomas, further supporting the role of epigenetic deregulation in gliomagenesis.[31-34] Accordingly, using Illumina 450K array-based methylation profiling, a novel subclassification of glioblastoma into 6 subgroups was proposed across age groups: the first three are linked to mutations of *IDH* or codons 27 or 34 of histone H3, which are mutually exclusive, the other groups were labelled receptor tyrosine kinase (RTK) I/PDGFRA, mesenchymal, and RTKII/classic. These six subclasses exhibited distinct profiles of age distribution, tumour localization, and outcome.[34] Importantly, this subclassification allowed to further split the proneural expression subgroups into basically four subgroups: *IDH*-mutant, *H3F3A* (K27)-mutant, *H3F3A* (G34)-mutant, and RTKI/PDGFRA. This is important since only the *IDH* group of proneural glioblastomas remains to be associated with a favourable prognosis, whereas the remaining patients do

as poorly as or even worse than patients with mesenchymal, classic, or neural tumours. The clinical usefulness of these novel classifiers is currently being tested in prospective cohort studies.

Molecular classification of other primary brain tumours

Ependymal tumours

Ependymomas remain a domain for surgical and radiooncological treatment approaches whereas pharmacological strategies have remained largely disappointing, notably in adults.[59] Although there has been significant progress in the molecular characterization of these tumours as well,[60] this has not resulted in the definition of promising new molecular targets for intervention yet. However, it has become evident that there is considerable heterogeneity within this group of tumours as well, and based on the fact that especially distinguishing between WHO grade II and grade III might very challenging from a neuropathology perspective, approaches to molecularly subclassify this disease based on published literature are currently being assessed.[61,62] For example, three prognostically relevant molecular subgroups of intracranial ependymomas have been proposed on the basis of DNA copy number changes: group 1 tumours have a favourable prognosis and carry gains on chromosomes 9, 15q and 18 or loss of chromosome 6; group 2 tumours have an intermediate prognosis and largely balanced genomic profiles; group 3 tumours have a poor prognosis and are characterized by 1q gains or homozygous cyclin-dependent kinase inhibitor 2A (*CDKN2A*) deletions.[60] Posterior fossa ependymomas have been reported to comprise three genetic subgroups characterized by (i) multiple concurrent DNA amplifications, (ii) gain of 1q, or (iii) a balanced karyotype. Moreover, ependymomas arising in different CNS regions, spinal, infratentorial or supratentorial, showed distinct mRNA expression signatures.[63] More recent data suggest two distinct subgroups of posterior fossa ependymomas based on gene expression profiles: group A tumours with poor prognosis with frequent relapse and metastatic seeding are preferentially found in younger patients and more common in males. These are typically located in lateral parts of the 4th ventricle and carry mostly balanced genomes with frequent gain of 1q and loss of 22. Group B tumours show a more favourable prognosis

and are genetically more unstable. These tumours are more prevalent in adults and located in the midline.[64] From a diagnostic perspective it is interesting that these two posterior fossa ependymoma subgroups may be distinguished immunohistochemically, with group A tumours expressing LAMA2 but not NELL2, while group B tumours show the opposite staining pattern.[64.65]

Medulloblastoma

Medulloblastoma is the most common malignant pediatric brain tumour, but may also occur in younger adults. More than any other brain tumour, medulloblastoma has become paradigmatic for the power of modern high throughput technology to allow subclassification and assignment to putative oncogenic pathways presumably reflecting different cells of origin or stages of neural development. Current approaches define four subgroups which, however, may be further subclassified: wingless (WNT), sonic hedgehog (SHH), group 3, and group 4, each characterized by differential expression profiles and characteristic patterns of age of onset, localization and outcome.[66-69] Most importantly, the pediatric neurooncology community came up with a consensus paper supported by leading groups across the world agreeing to this classification approach.[70] An immediate clinical consequence of this ground-breaking work has been that most new studies in North America and Europe account for the fact that WNT-driven medulloblastoma patients have an excellent overall survival with current therapy regimens, so it is now being tested whether it is safe to reduce the dose of RT for these patients. The first clinical experience with the smoothened inhibitor vismodegib as a targeted approach to medulloblastoma demonstrated a dramatic, albeit short-lived response.[71] However, larger studies on less-heavily pre-treated patients are forthcoming using either vismodegib or LDE-225 and at first glance show promising results in patients whose tumours carry a SHH signature. A French-led European trial (MEVITEM, www.clinicaltrials.gov NCT#01601184) compares temozolomide alone versus temozolomide plus vismodegib in adult patients with recurrent medulloblastoma with SHH signature. Other approaches for the implementation of targeted therapeutic approaches into the pediatric and adult medulloblastoma clinical trial portfolio based on novel molecular classifiers are ongoing.

Meningioma

Meningiomas are the most common tumors among all primary brain tumours. Although they are histologically benign most often (>90%) and histological subgroups have little clinical significance, a minority of meningiomas shows histological features of atypia, including most notably elevated mitotic activity or brain-infiltrative growth patterns. These atypical meningiomas correspond to WHO grade II and are associated with a high likelihood of local recurrence even after macroscopically complete resection. The rare anaplastic meningiomas (WHO grade III) are fast growing tumours with locally destructive growth that may even produce systemic metastases, mostly in lung, liver and bone (Webappendix). Thus, meningiomas represent a major challenge in neurological oncology. Surgery and RT are the principal therapeutic measures. Systemic pharmacotherapy has been notoriously unsuccessful, and molecular genetic profiling has not provided clues for targeted therapy approaches.[72] For decades there has been major interest in hormonal therapies for these tumours because estrogen receptors are expressed in approximately 10% and progesterone receptors in more than half of meningiomas, but therapeutic targeting of hormone receptors has not been successful. Somatostatin receptors may be expressed in 80-90% of meningiomas. Their assessment using immunohistochemistry or positron emission tomography (PET) has been proposed to select patients for treatment with somatostatin receptor agonists, but a recent prospective study exploring the activity of octreotide yielded disappointing results, with no responses and only 2 of 12 patients with prolonged stable disease.[73] Genomic profiling has revealed inactivation of the tumour suppressor gene *NF2* on 22q in about 50% of meningiomas, including the majority of transitional, fibroblastic, atypical and anaplastic meningiomas. More recently, recurrent oncogenic mutations have been discovered in *SMOH* and *AKT1* by deep sequencing approaches in *NF2* wildtype tumours including special histological subtypes like the secretory meningioma.[74] Further, genome-wide analyses of NF2-wildtype meningiomas do not only point towards different genetic meningioma entities, but also revealed potential novel targets for intervention. Almost 25% of the meningiomas showed partly recurrent mutations in TNF receptor-associated factor (TRAF) 7, a protein involved in signalling processes of differentiation and apoptosis. *SMOH* mutations, which activate Hedgehog signalling, were identified in ~5% of NF2 wildtype meningiomas. These tumours, which were mainly of WHO

grade I, usually showed stable genomes, whereas atypical or anaplastic meningiomas often carried *NF2* mutations, unstable genomes and demonstrated a predominantly hemispheric localization.[75,76]

Outlook

Tremendous progress has been made in the molecular classification of primary brain tumours. In the case of *MGMT* promoter methylation in glioblastoma in the elderly and 1p/19q co-deletions in anaplastic oligodendroglial tumours, molecular markers determine clinical decision making as of 2012, based on few practice-changing academic trials.[13,14,24,25] Depending on the outcome of ongoing phase II and III trials of novel targeted agents in newly diagnosed and recurrent glioblastoma, biomarkers to predict resistance or sensitivity to angiogenesis inhibition will move into focus, both in tumour tissue and in peripheral blood. In medulloblastoma, molecular subclassification is now used for selecting targeted agents depending on the dominant oncogenic pathway. Meanwhile, high-throughput analyses at genetic, epigenetic and expression levels have demonstrated their value in classifying brain tumours and prognosticating outcome. These techniques may soon become more widely available, easier to standardise and less subject to bias, than single marker assessments, e.g., current ways of determining the *MGMT* status, and may soon become more cost-effective, too. Accordingly, we predict that the current histology-dominated diagnostic assessment of brain tumours will be increasingly supplemented by molecular diagnostic tests, which eventually may be gradually replaced by high throughput profiling techniques, including array-based approaches and next-generation sequencing. This progress in molecular diagnostics will help to improve the precision of histological diagnoses, to select appropriate therapeutic measures, and to enrich patient populations for clinical trials.

Yet, it is also important to realize that array-based approaches will not completely supplant targeted analyses. There are still instances where diagnoses are being rendered on miniscule portions of tissue obtained by biopsy or from the very edge of infiltrating gliomas where the nature of the lesion, tumour or not, is uncertain, and high-throughput techniques might not be as helpful or simply cannot be

applied due to limited tissue availability.

In addition to the complex interdisciplinary dialogue required for optimized clinical decision making, the technical challenges associated with assessing molecular markers in brain tumour patients further support the call for centralized care of patients with relatively rare tumours, for whom an increasing repertoire of novel treatment options is currently being made available.

Contributor statement

MW prepared the first draft of the manuscript. SMP, WW, MEH, GR and RS reviewed and revised the manuscript. All authors approved the final version of the manuscript.

Conflicts of interest

MW has received research grants from Antisense Pharma, Merck Serono and Roche and honoraria for lectures or advisory boards from Antisense Pharma, Magforce, Merck Serono, MSD and Roche. He was the principal investigator of phase II or phase III trials investigating temozolomide (MSD) in newly diagnosed anaplastic glioma and glioblastoma as well as recurrent glioblastoma.

SMP has received honoraria for lectures or advisory boards from Novartis.

WW has received research grants from Eli Lilly, MSD and Roche and honoraria for lectures or advisory boards from Magforce, MSD and Roche. He is the principal investigator of phase II trials investigating an oral TGF-β receptor inhibitor (JBAI and JBAL, Eli Lilly) and APG101 (Apogenix) in glioblastoma.

MEH has received research grants from AstraZeneca, and is an advisor to MDxHealth, and EMD-Serono and has received honoraria for advisory boards from MSD and Roche.

GR has received honoraria for advisory boards from Merck Serono.

RS served on advisory boards to MSD (Merck & Co.), Merck Serono (EMD), and Roche/Genentech. He is the principal investigator of phase III trials investigating Cilengitide (Merck Serono) and the NovoTTF device (Novocure Ltd) in glioblastoma.

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Figure legends

Figure 1. **Biomarker-based approach to anaplastic glioma**

Red: new standard practice, blue: to be confirmed: italics: alternative options.

Figure 2. **Biomarker-based approach to glioblastoma**

Red: new standard practice, italics: alternative options.

Table 1. Molecular markers in gliomas: biological role, assessment, and clinical value.

	***IDH1/2* mutation**	**1p/19q co-deletion**	***MGMT* promoter methylation**	***EGFRvIII* rearrangement**	***BRAF* duplication / fusion**	***BRAF* activating point mutation**
Biological consequence	Increased levels of 2-hydroxyglutarate, link to G-CIMP phenotype	Unclear, candidate genes *CIC* and *FUBP1* under investigation	Reduced DNA repair, association with G-CIMP phenotype in *IDH*1/2-mutant tumors	Ligand-independent pathway activation	MAPK pathway activation	MAPK pathway activation
Methods of assessment	Immunohistochemistry for IDH1-R132H, (pyro)sequencing	FISH, microsatellite analysis for loss of heterozygosity	MSP, MS or bisulfite (pyro)sequencing	RT-PCR, immunohistochemistry, MLPA	FISH, RT-PCR	Immunohistochemistry for BRAF-V600E, (pyro)sequencing
Incidence						
Pilocytic astrocytoma	0%	0%	<10%	0%	50-70%	10%
Pleomorphic xanthoastrocytoma	0%	0%	10-20%	0%	rare	60-70%
Diffuse astrocytoma	70-80%	15%	40-50%	0%	rare	rare
Oligodendroglioma/ oligoastrocytoma	70-80%	30-60%	60-80%	0%	rare	rare
Anaplastic astrocytoma	50-70%	15%	50%	0%	rare	rare
Anaplastic oligodendroglioma/ oligoastrocytoma	50-80%	50-80%	70%	0%	rare	rare
Glioblastoma	5-10%	<5%	35%	25-30%	rare	3-5%

Diagnostic role	Important, see above, differential diagnosis between diffuse glioma and gliosis	Strong association with oligodendroglial morphology, differential diagnosis of brain tumors with clear cells	None	Strong association with glioblastoma	Yes, see above	Yes, see above
Prognostic role	Positive across histologies	Favorable for oligodendroglial tumors treated with RT or alkylating agent chemotherapy or both	Prognostic for anaplastic glioma patients (? with *IDH* mutations) treated with RT or alkylating agents	Negative prognostic factor, reduced long-term survival	unclear	unclear
Predictive role	Absence of mutation suggests predictive role for *MGMT* promoter methylation	Patients with 1p/19-codeleted (anaplastic) oligodendrogliomas should not be treated with RT alone, but alkylating agents ± RT	Predictive for glioblastoma (? without *IDH* mutations) treated with alkylating agents, should be tested in elderly glioblastoma patients	Possible biomarker for vaccination	Possible biomarker for targeted therapy	Possible biomarker for targeted therapy

Table 2. Outcome by 1p/19q codeletion status in the anaplastic oligodendroglioma trials.[13,14]

	EORTC 26951 (n=368)			**RTOG 9402 (n=291)**		
	RT	**RT→PCV**		**RT**	**PCV→RT**	
All patients						
Progression-free survival (years)	1.1	2.0	HR=0.66 95% CI 0.52-0.83	no data in 2013 update	no data in 2013 update	
Overall survival (years)	2.5	3.5	HR=0.75 95% CI 0.6-0.95	4.7	4.6	HR=0.79 95% CI 0.6-1.04
Patients with 1p/19q-codeleted tumors						
Progression-free survival (years)	4.2	13.1	HR=0.42 95% CI 0.24-0.74	2.9	8.4	HR=0.47 95% CI 0.3-0.72
Overall survival (years)	9.3	Not reached	HR=0.56 95% CI 0.31-1.03	7.3	14.7	HR=0.59 95% CI 0.37-0.95
Patients with 1p/19q-non-codeleted tumors						
Progression-free survival (years)	0.7	1.2	0.73 95% CI 0.56-0.97	1	1.2	HR=0.81 95% CI 56-116
Overall survival (years)	1.8	2.1	HR=0.83 95% CI 0.62-1.1	2.7	2.6	HR=0.85 95% CI 0.58-1.23

Table 3. Outcome by *MGMT* promoter methylation status in the elderly glioblastoma (anaplastic astrocytoma) trials.[24,25]

	NOA-08[1]			Nordic trial			
	RT 30 x 2 Gy (n=178)	**TMZ 7/7 (n=195)**		**RT 30 x 2 Gy (n=100)**	**RT 10 x 3.4 Gy (n=123)**	**TMZ 5/28 (n=119)**	
All patients							
Progression-free survival (months)	4.7	3.3	HR=1.15 95% CI 0.92-1.43 $p_{\text{non-inferiority}}$=0.043		not reported		
Overall survival (months)	9.6	8.6	HR=1.09 95% CI 0.84-1.42 $p_{\text{non-inferiority}}$=0.033	6	7.5	8.3	[3]TMZ: HR=0.70 95% CI 0.52-0.93 p=0.01 [3]Hypofractionated RT: HR=0.85 95% CI 0.64-1.12 p=0.24
Patients with *MGMT* promoter-methylated tumours							
Progression-free survival (months)	4.6	8.4	HR=0.53 95% CI 0.33–0.86 p=0.01		not reported		
Overall survival (months)	9.6	not reached	HR=0.69 95% CI 0.35–1.16 p=0.14		8.2[2]	9.7	HR=0.64 95% CI 0.39-1.04
Patients with *MGMT* promoter-unmethylated tumours							

Progression-free survival (months)	4.6	3.3	HR=1.95 95% CI 1.41–2.69 p=0.01		not reported		
Overall survival (months)	10.4	7	HR=1.34 95% CI 0.92–1.95 p= 0.13		7[1]	6.8	HR=1.16 95% CI 0.78-1.72

[1]comprised 11% anaplastic astrocytoma
[2]both RT groups pooled
[3]comparison to standard RT (30 x 2 Gy)
[4]TMZ relative to all patients receiving RT (with or without *MGMT* promoter methylation) which were pooled because they had a similar outcome in NOA-08

Figure 1

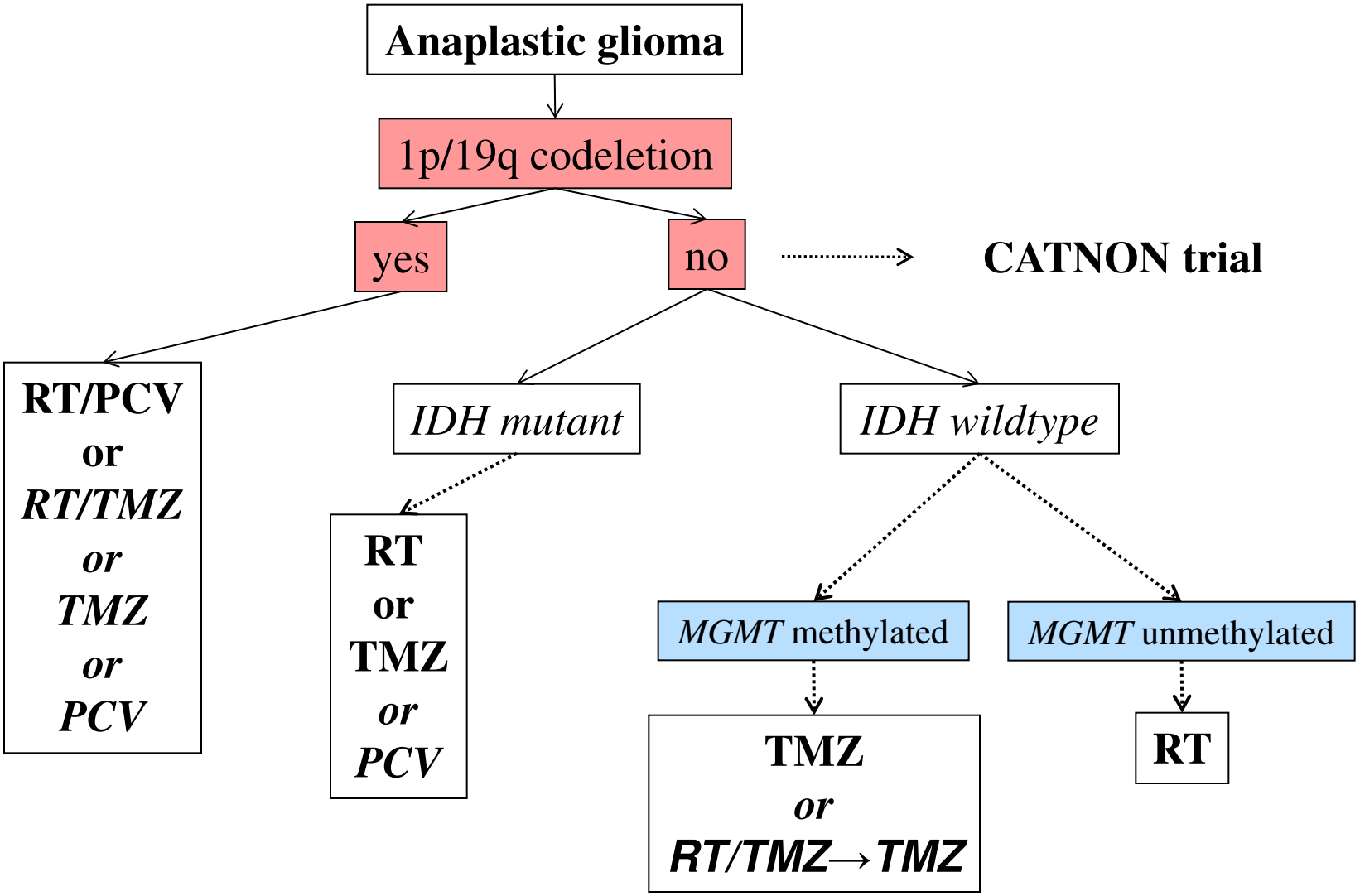
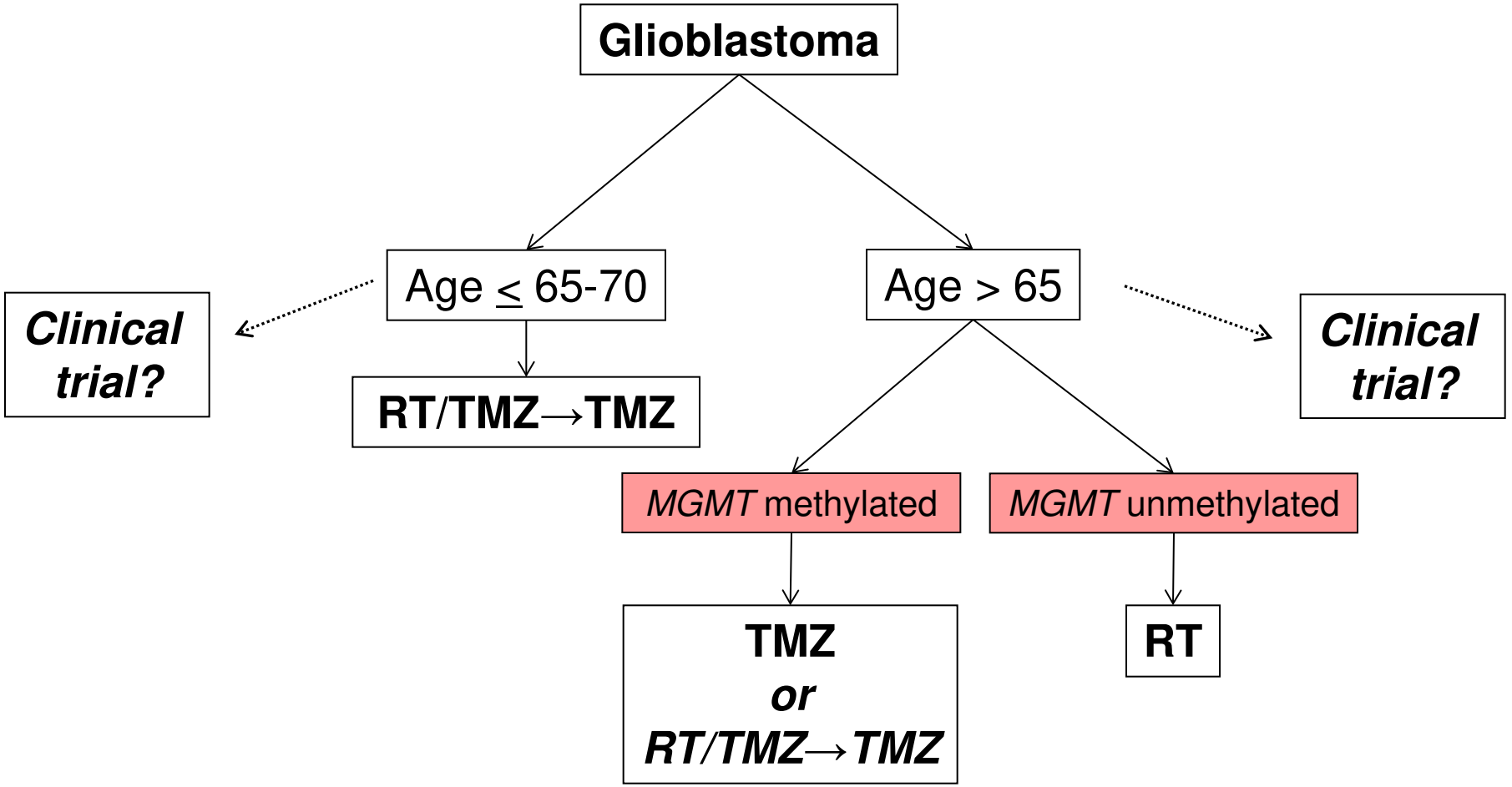

Anaplastic glioma
1p/19q codeletion
yes
no
CATNON trial
RT/PCV
or
RT/TMZ
or
TMZ
or
PCV
IDH mutant
IDH wildtype
RT
or
TMZ
or
PCV
MGMT methylated
MGMT unmethylated
TMZ
or
RT/TMZ→TMZ
RT

Figure 2


Glioblastoma
Age ≤ 65-70
Age > 65
Clinical trial?
RT/TMZ→TMZ
Clinical trial?
MGMT methylated
MGMT unmethylated
TMZ or RT/TMZ→TMZ
RT